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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/978,498	10/15/2001	Adrian Clausell	2055-181	4848

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EXAMINER
PRATS, FRANCISCO CHANDLER

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 09/15/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/978,498	CLAUSELL ET AL.	
	Examiner	Art Unit	
	Francisco C Prats	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 July 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *5-8,11-14,16-20 and 23-53* is/are pending in the application.

4a) Of the above claim(s) *23-37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5-8, 11-14, 16-20 and 38-53* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 12, 2004, has been entered.

Claims 5-8, 11-14, 16-20 and 23-53 are pending.

Election/Restrictions

Claims 23-37 remain withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected species of invention, there being no allowable generic or linking claim. As discussed in the previous office action, election was made **without** traverse in Paper No. 8 filed May 21, 2003.

Claims 5-8, 11-14, 16-20 and 38-53 are examined on the merits to the extent they read on the elected species (caspase, glycerol, rhodamine 110), as well as the use of DMSO as an uptake-enhancing agent for intact cell enzyme assays.

Claim Rejections - 35 USC § 103

Claims 5, 11, 12 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lucas et al (U.S. Pat. 5,698,411).

Lucas discloses the use of the derivates of elected species of indicator moiety, rhodamine 110, in assays of whole cell enzyme activity. Lucas discloses that additional agents, such as DMSO, preferably at 5%, may be used to assist the transfer of the assay compounds into cells. See column 29, line 61 through column 30, line 40. As amended, claim 5 and its dependents now require the substrate/analyte/enhancer solution to be added to the cells to be analyzed in a manner such that the solution forms a layer over the intact metabolic cells. Lucas' addition of the substrate/analyte/enhancer solution to the intact metabolically active cells inherently results in cells covered, at least in part, by the substrate/analyte/enhancer solution. The cells can therefore be considered to be covered by "a layer" of said solution. If there were no layer of solution over the cells, then the substrate could not enter the cells. Lucas is therefore considered to meet this limitation.

Lucas differs from the claimed subject matter in failing to explicitly disclose a single embodiment combining the uptake-enhancing agent with multiple enzyme assays, either simultaneous

or sequential, as recited in claims 11 and 12, or the use of 20 to 60% DMSO as the solubilizing agent.

However, Lucas clearly discloses that adequate analysis of the disease states of cells requires multiple enzyme assays, with the generation of a specific data matrix for a number of different enzymes. See discussion at columns 43-48. The artisan of ordinary skill, recognizing that the enzyme assays would have been suitably performed either sequentially or simultaneously, clearly would have been motivated to have performed the assays using either tactic, reasonably expecting to generate the required data set. Thus, the claimed combination of uptake-enhancing agent with multiple enzyme assays clearly would have been obvious in view of Lucas' disclosure, the artisan of ordinary skill recognizing the advantages of solubility agents as disclosed by Lucas, and also recognizing the suitability of multiple enzyme assays to generate the data set disclosed by Lucas as being required for accurate disease diagnosis. A holding of obviousness over claims 11 and 12 is therefore required.

As to the amount of DMSO recited in the claims, note specifically that Lucas clearly discloses that suitable amounts of solubilizing agent can be determined by optimization. See column 30, lines 13 and 14 ("The effective amount of

solubilizing component may be empirically determined . . . ."). Because Lucas considers the concentration of solubilizing component to be a result-effective parameter which can be routinely optimized, the claimed concentration of DMSO must be considered obvious absent some demonstration that said concentration confers an unexpected result upon the claimed subject matter. A holding of obviousness is therefore required over claim 22.

All of applicant's argument regarding this ground of rejection has been fully considered but is not persuasive of error. While applicant asserts that the use of a non-homogenous assay mixture is not taught by Lucas, it is respectfully submitted that the claims, even as amended, are sufficiently broad to encompass the processes suggested by Lucas. Specifically, when one mixes intact cells with an assay solution as disclosed by Lucas, the result is that the cells will be covered, at least in part, by the assay solution, even if the cells are suspended in the solution. Thus, the current recitation requiring a layer of solution over the cells does not serve to distinguish the claims over Lucas.

As to the use of higher amounts of DMSO recited in the claims as amended, note specifically, as stated above and in the previous office action, that suitable amounts of solubilizer

would have been readily determined through routine experimentation. While applicant points to the Lucas reference's discussion about problems with higher concentrations of solubilizers, applicant's argument fails to point out that Lucas provides methods for solving this problem. See column 30, lines 55, et seq., discussing how the problem of cellular expulsion can be solved by adding other ingredients.

Claims 5-8, 11-14, 20 and 38-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Landrum et al (U.S. Pat. 5,976,822) in view of Lucas et al (U.S. Pat. 5,698,411).

Landrum discloses the use of derivates of the elected species of indicator moiety, rhodamine 110, in assays of whole cell enzyme activity, including caspase activity, for the purpose of ascertaining apoptotic cells, as well as for the purpose of distinguishing apoptotic cells from necrotic cells. See Example 10, at column 22, line 34 through column. See also, abstract. Note specifically the use of different substrate moieties for different enzymes, disclosed at Table 1, column 22, lines 1-19. As amended, claim 5 and its dependents now require the substrate/analyte/enhancer solution to be added to the cells to be analyzed in a manner such that the solution forms a layer over the intact metabolic cells. Landrum's addition of the

substrate/analyte/enhancer solution to the intact metabolically active cells inherently results in cells covered, at least in part, by substrate/analyte/enhancer solution. The cells can therefore be considered to be covered by "a layer" of said solution. If there were no layer of solution over the cells, then the substrate could not enter the cells. Landrum is therefore considered to meet this limitation.

Landrum differs from the claims in that Landrum does not disclose the use of uptake-enhancing agents in the assays. However, Landrum clearly discloses that additional ingredients, including "solubilizing components" can be used to improve the assays conducted according to the disclosure therein. See column 10, lines 21-37. Moreover, Lucas clearly discloses that intact cell enzyme assays using rhodamine 110 derivatives can benefit from the addition of solubilizing agents which allow the assay compound to pass into the cell. See column 29, line 61 through column 30, line 40. Thus, the artisan of ordinary skill at the time of applicant's invention clearly would have recognized from Lucas the advantages of solubilizing agents in assays using rhodamine 110 derivatives as assay compounds. The artisan of ordinary skill would therefore have been motivated to have used Lucas' solubilizing compounds in the caspase assays of Landrum which also use rhodamine 110 as the assay compound,

thereby assisting in the transfer of the assay compound into the intact cells, as disclosed by Lucas. A holding of obviousness over the cited claims is therefore required.

All of applicant's argument regarding this ground of rejection has been fully considered but is not persuasive of error. Applicant urges that because the assays in Landrum and Lucas were conducted in homogeneous assay mixtures, neither of these references discloses or suggests the non-homogeneous assay mixtures recited in the claims as amended, which require the assay solution to be in a layer over the cells. However, it is respectfully submitted that the present claims require only a "layer" of assay solution over the cells. As pointed out above, when one mixes intact cells with an assay solution as disclosed by Lucas, the result is that the cells will be covered, at least in part, by the assay solution, even if the cells are suspended in the solution. The solution covering the cells can be considered a "layer," and that is all that the claims require. In sum, the current recitation requiring a layer of solution over the cells does not serve to distinguish the claims over Lucas or Landrum. Moreover, in view of the clear suggestion to optimize the amount of permeabilizing agent, the claimed concentration of DMSO must be considered obvious absent some demonstration of an unexpected result coming therefrom.

Claims 5-8, 11-14, 20 and 38-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (U.S. Pat. 6,248,904 B1).

Zhang discloses the use of derivates of the elected species of indicator moiety, rhodamine 110, in assays of whole cell enzyme activity, including caspase activity, for the purpose of ascertaining apoptotic as well as anticancer efficacy of therapeutic agents. Zhang discloses that additional solubilizing agents, such as DMSO, as well as liposomes or detergents, may be used to assist the transfer of the assay compounds into cells. See column 39, lines 48-64.

As amended, claim 5 and its dependents now require the substrate/analyte/enhancer solution to be added to the cells to be analyzed in a manner such that the solution forms a layer over the intact metabolic cells. Zhang's addition of the substrate/analyte/enhancer solution to the intact metabolically active cells inherently results in cells covered, at least in part, by substrate/analyte/enhancer solution. The cells can therefore be considered to be covered by "a layer" of said solution. If there were no layer of solution over the cells, then the substrate could not enter the cells. Particular motivation for the "layered" arrangement recited in the claims

is Zhang's disclosure that the assays may be performed on "cells grown in culture in form of a monolayers" (column 55, lines 4-5), an arrangement which would inherently result in a layer of assay medium over the monolayer of cells. Zhang is therefore considered to meet the limitation requiring a layer of medium over the cells.

Zhang differs from the claims in failing to explicitly disclose a single embodiment combining the uptake-enhancing agent with multiple enzyme assays, either simultaneous or sequential, or the use of 20 to 60% DMSO as the solubilizing agent.

However, Zhang clearly discloses that different disease states can be assayed using different enzyme assays. See discussion at column 5, line 4 through column 6, line 15. The artisan of ordinary skill, recognizing that the enzyme assays for disease diagnosis would have been suitably performed either sequentially or simultaneously, clearly would have been motivated to have performed the assays using either tactic, reasonably expecting to generate the disease diagnosis regardless of whether the assays were performed at the same time, or one after the other. Thus, the claimed combination of uptake-enhancing agent with multiple enzyme assays clearly would have been obvious in view of Zhang's disclosure, the artisan of

ordinary skill recognizing the advantages of solubility agents as disclosed by Zhang's, and also recognizing the suitability of multiple enzyme assays to generate the data set disclosed by Zhang as being required for accurate disease diagnosis.

As to the amount of DMSO recited in the claims, the artisan of ordinary skill practicing Zhang's methods clearly would have recognized that using differing concentrations of solubilizing component would have resulted in different results. Thus, the artisan of ordinary skill would have considered the concentration of solubilizing component to be a result-effective parameter which would have been routinely optimized. The claimed concentration of DMSO must be considered obvious absent some demonstration that said concentration confers an unexpected result upon the claimed subject matter. A holding of obviousness is therefore required over claims.

Further still, assays of intensity or magnitude over time were well known in the art at the time of applicant's invention. Absent some demonstration that these assays perform in a manner unexpected in view of the prior art, a holding of obviousness over the cited claims is clearly required.

In sum, because the prior art fairly suggests the claimed subject matter, a holding of obviousness is clearly required.

All of applicant's argument regarding this ground of rejection has been fully considered but is not persuasive of error. Applicant urges that because the assays in Zhang were conducted in homogeneous assay mixtures, neither of these references discloses or suggests the non-homogeneous assay mixtures recited in the claims as amended, which require the assay solution to be in a layer over the cells. However, it is respectfully submitted that the present claims require only a "layer" of assay solution over the cells. As pointed out above, when one mixes intact cells with an assay solution as disclosed by Zhang, the result is that the cells will be covered, at least in part, by the assay solution, even if the cells are suspended in the solution. The solution covering the cells can be considered a "layer," and that is all that the claims require. Moreover, as discussed above, Zhang clearly discloses that the assayed cells can be in the form of a cultured monolayer, an arrangement which would clearly result in the layered assay configuration presently recited the claims as now amended. In sum, the current recitation requiring a layer of solution over the cells does not serve to distinguish the claims over Lucas or Landrum. Moreover, in view of the clear suggestion to optimize the amount of permeabilizing agent, the claimed concentration of

DMSO must be considered obvious absent some demonstration of an unexpected result coming therefrom.

Claims 5-8, 11-14, 16-20 and 38-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Landrum et al (U.S. Pat. 5,976,822) in view of Lucas et al (U.S. Pat. 5,698,411), as applied to claims 5-8, 1-14, 20 and 38-53 above, and in further view of Wansink et al (J. Cell Biol. 122(2):283-293 (1993)).

The claims under examination now positively recite embodiments requiring the use of glycerol as a permeabilizing agent in caspase assays, in assays using rhodamine 110, and in caspase assays using rhodamine 110. As discussed above with respect to Landrum and Lucas, these references suggest the use of permeabilizing agents such as DMSO for the purpose of allowing an assay indicator compound to enter the cell in whole-cell enzyme assays. Neither Landrum nor Lucas disclose the use of glycerol as the permeabilizing agent.

However, Wansink et al disclose a process whereby incorporation of BrUTP into RNA is measured over time in permeabilized human bladder carcinoma cells. See Fig. 1, page 285. The cells were permeabilized using a buffer comprising 25% glycerol, the elected species of uptake-enhancing agent. See page 284, left hand column, section entitled "*BrUTP*

Incorporation in Permeabilized Cells (Run-on Transcription)", subsection entitled "*Cells in Suspension.*" Thus, the artisan of ordinary skill, recognizing from Wansink that 25% glycerol was a permeabilizing agent suitable for allowing entry of indicator compounds into intact cells for whole-cell assays, clearly would have been motivated to have used said glycerol in the assays disclosed by Landrum and/or Lucas.

All of applicant's argument regarding this ground of rejection has been fully considered but is not persuasive of error. For the reasons discussed above, Landrum and Lucas are considered to suggest the "layer" arrangement of assay medium, because any addition of assay medium will produce a layer of medium covering, or "over," the cells. Moreover, while it is noted that Wansink's assay ultimately destroys the cells assayed, unlike the Lucas/Landrum assays, the simple fact is that Wansink discloses that glycerol is a cell-permeabilizing agent which allows the cells to retain metabolic activity. Thus, the artisan of ordinary skill practicing the Lucas/Landrum methods with permeabilizing agents clearly would have recognized from Wansink the suitability of glycerol as a cell-permeabilizing agent, and would have been motivated by the disclosed suitability to have used glycerol in the Lucas/Landrum

methods. It is therefore respectfully submitted that a holding of obviousness remains required.

Claims 5-8, 11-14, 16-20 and 38-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (U.S. Pat. 6,248,904 B1), as applied to claims 5-14, 20 and 38-53 above, and in further view of Wansink et al (J. Cell Biol. 122(2):283-293 (1993)).

The claims under examination now positively recite embodiments requiring the use of glycerol as a permeabilizing agent in caspase assays, in assays using rhodamine 110, and in caspase assays using rhodamine 110. As discussed above with respect to Zhang, that reference suggests the use of permeabilizing agents such as DMSO for the purpose of allowing an assay indicator compound to enter the cell in whole-cell enzyme assays. Zhang does not disclose the use of glycerol as the permeabilizing agent.

However, Wansink et al disclose a process whereby incorporation of BrUTP into RNA is measured over time in permeabilized human bladder carcinoma cells. See Fig. 1, page 285. The cells were permeabilized using a buffer comprising 25% glycerol, the elected species of uptake-enhancing agent. See page 284, left hand column, section entitled "*BrUTP*

Incorporation in Permeabilized Cells (Run-on Transcription)", subsection entitled "*Cells in Suspension.*" Thus, the artisan of ordinary skill, recognizing from Wansink that 25% glycerol was a permeabilizing agent suitable for allowing entry of indicator compounds into intact cells for whole-cell assays, clearly would have been motivated to have used said glycerol in the assays disclosed by Zhang.

All of applicant's argument regarding this ground of rejection has been fully considered but is not persuasive of error. For the reasons discussed above, Zhang is considered to suggest the "layer" arrangement of assay medium, because any addition of assay medium will produce a layer of medium covering, or "over," the cells, and because assaying monolayers of cells as suggested by Zhang would result in the claimed layered assay configuration. Moreover, while it is noted that Wansink's assay ultimately destroys the cells assayed, unlike the Zhang assays, the simple fact is that Wansink discloses that glycerol is a cell-permeabilizing agent which allows the cells to retain metabolic activity. Thus, the artisan of ordinary skill practicing the Zhang methods with permeabilizing agents clearly would have recognized from Wansink the suitability of glycerol as a cell-permeabilizing agent, and would have been motivated by the disclosed suitability to have used glycerol in

the Zhang methods. It is therefore respectfully submitted that a holding of obviousness remains required.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Francisco C Prats whose telephone number is 571-272-0921. The examiner can normally be reached on Monday through Friday, with alternate Fridays off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Francisco C Prats
Primary Examiner
Art Unit 1651

FCP